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Investigation on clonal variants within the hazelnut (*Corylus avellana* L.) cultivar ‘Tonda Gentile delle Langhe’

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Abstract:

The hazelnut cultivar ‘Tonda Gentile delle Langhe’ (TGdL) from Piedmont (Northwest Italy) is known worldwide for the excellent quality of its nuts and is thought to have a monoclonal origin. During 3 years of *on-farm* exploration carried out in Piedmont within the AGRI-GEN-RES project SAFENUT, 6 accessions of a minor cultivar called ‘Tonda di Biglini’ (TdB), known for its nut quality similar to TGdL, and 2 plants with low or null suckering habit, found in an orchard of TGdL, were characterized with TGdL reference plants using molecular markers and plant descriptors. In addition, agronomical behaviour and nut quality of TdB were evaluated in a field trial aimed at comparing TdB and TGdL in the same environmental conditions. Although analyses at 27 SSR hazelnut loci revealed the same genetic profile of TGdL for all the considered accessions, observations showed relevant differences in phenological and nut traits: earlier female flowering and nut maturity times (10 days) in TdB than in TGdL; lower percent kernel and higher presence of double kernels in TdB than in TGdL. These results were confirmed in the field trial comparison and were observed also in the non-suckering plants. A sensory analysis conducted on TGdL and TdB kernels using triangle test gave a significant identification indicating organoleptic differences between the 2 cultivars. In conclusion, given that the distinctive characters found are stable and maintained through propagation as shown in the field trial, TdB can indeed be considered a different cultivar while for the non-suckering individuals the stability of the traits after propagation still need to be confirmed. This report demonstrates the presence of mutations of agronomical relevance within a monoclonal cultivar of hazelnut.

Keywords: Tonda di Biglini, phenological traits, nut quality, molecular markers, sensory analysis, mutation

1. Introduction

Italy is the second worldwide hazelnut producer with 113,200 t and 68,000 ha invested (means 2007-2011, FAOstat). The 98% of the producing surface is located in four regions: Campania, Latium, Piedmont, and Sicily. The Piedmont region (North-West Italy) accounts for 18% of the total production area that is constantly expanding; in the last ten years the planted surface rose by more than 50%, from 8,042 ha in 2001 to 12,133 ha in 2011 (ISTAT). Nut production is 1,650 t (means 2007-2011; ISTAT) and is based on a single cultivar: ‘Tonda Gentile delle Langhe’ (TGdL). It is also known since the end of XIX century as ‘Tonda Gentile del Piemonte’ or ‘Ronde du Piémont’ and has been investigated since the 50s of the XX century (Carlone, 1957; Romisondo, 1960). TGdL was selected directly by farmers for its good environmental adaptability to Piedmont climate and for the excellent quality of the kernel. It is internationally known and widely used by the food industry for the high quality of its nuts, particularly suitable for processing. The cultivar was already exported in many foreign countries, including Japan and Chile, since the beginning of XX century (Romisondo et al., 1983). The traditional cultivation area of TGdL is mainly located on the hills of the Langhe district (Cuneo province), where it grows in the absence of pollinators due to the abundant presence of wild hazelnuts in the woods. This situation and the high commercial value of the production (still the best paid by the industry worldwide), has made TGdL the only cultivar grown in Piedmont. Recently, for commercial reasons, the name of the cultivar was changed in ‘Tonda Gentile Trilobata’ referring to the trilobate shape of the nut. ‘Tonda Gentile Trilobata’ is protected by the European Union under the Geographical Indication (PGI) ‘Nocciola Piemonte’. In this paper we will use the name ‘Tonda Gentile delle Langhe’ which is best known worldwide.

TGdL is considered to have a monoclonal origin and is propagated clonally by mound layering or rooted suckers. Yet, some clonal variability has been observed across years. As for other fruit and nut crops, clonal selection was carried out in the '70 and '80 (Romisondo et al., 1979); studies showed a certain degree of morphological and phenological variability within the population of individuals but as well were not able to distinguish genetically the selected clones using RAPD markers (Valentini et al., 2001).

During three consecutive years (2007-2009) the EU AGRI GEN RES project SAFENUT (‘Safeguard of almond and hazelnut genetic resources: from traditional uses to modern agro-industrial opportunities’) aimed at increasing knowledge of genetic diversity in the European hazelnut. Objectives included description

of cultivars from different *ex situ* European collections as well as the *on-farm* exploration, description, and *in situ* conservation of local cultivars and landraces. This characterization was carried out using different set of descriptors: morphological, biochemical, molecular, as well as ecological and cultural aspects. In Piedmont, the only cultivar of some importance surveyed was ‘Tonda di Biglini’ (TdB), a variety mentioned since the ‘60s and also known as ‘Grossa della Piana dei Biglini’ (Carlone, 1962; Fregoni and Zioni, 1964; Romisondo, 1960). It is mostly grown in Biglini, a suburb of the city of Alba in the Langhe hills (Cuneo province), and sporadically in surrounding areas. Although the quality of its nut is similar to that of TGdL, it has been considered a distinct cultivar from TGdL to which it is sometimes preferred for some agronomic characteristics, such as the remarkable precocity of fruit ripening and the high productivity. However, the cultivar has neither been accurately described nor evaluated for agronomic performance in comparison with TGdL, under the same pedoclimatic conditions.

In hazelnut, the current methods to characterize and identify cultivars are based on morphological and phenological descriptors, (Bioversity International, 2008; Thompson et al., 1978; UPOV, 1979) and molecular markers. Microsatellite or simple sequence repeat (SSR) markers are commonly recognised as markers of choice for cultivar characterisation in several plant species. In *C. avellana* SSR markers have been developed (Bassil et al., 2005a, 2005b; Boccacci et al., 2005; Gürcan and Mehlenbacher, 2010; Gürcan et al., 2010a) and used to fingerprint accessions in collections, identify synonyms, determine parentage, and assess genetic relationships among cultivars (Boccacci et al., 2006, 2008; Gökirmak et al., 2009; Gürcan et al., 2010b).

Aim of this research was the *on-farm* characterisation of ‘Tonda di Biglini’ (TdB) and of low and non suckering individuals of TGdL, rescued during the SAFENUT survey, using SSR markers, morphological and phenological descriptors, and sensory analysis. At the same time a field trial was set up aimed at comparing agronomical behaviour and nut quality of TdB and TGdL in the same environmental conditions.

2. Material and methods

2.1. Accessions surveyed on-farm

Six accessions of ‘Tonda di Biglini’ (TdB) were surveyed *on-farm* in three localities of the Cuneo province and labelled with a SAF code (Table 1). The accessions SAF5 and SAF6 were from Verduno, the SAF7 and SAF8 from Biglini, and the last two accessions, SAF11 and SAF12, from Lequio Berria. Two accessions of the cultivar ‘Tonda Gentile delle Langhe’ (TGdL) with low (SAF148) or absent (SAF149) suckering aptitude were surveyed in Castelletto Stura.

Five plants of TGdL cultivar were used as reference. One plant was located in Verduno (TGdL1) in the same orchard of SAF5 and SAF6 accessions, one (TGdL2) in Cravanzana, and one (TGdL3) in Tigliole (N 44°53’, E 08°04’, altitude 239 m a.s.l.). Other two clones of TGdL (TGdL4 and TGdL5) named UNITO-MT5 and UNITO-PD6 (Valentini et al., 2001), were located in an experimental field sited in Cravanzana.

2.2. *Morphological and phenological observations conducted on-farm*

Plants and nuts of SAF accessions were described *on-farm* using a set of descriptors (Bacchetta et al., 2009) selected and partially modified from those reported by Thompson et al. (1978), UPOV (1979) and Bioversity International (2008).

During 2007-2009 period the vegetative traits of the tree were described using five qualitative descriptors: vigour, habit, density of the shoots, suckering aptitude, and productivity. Husks or involucre, nuts and kernels were characterized using 11 qualitative standard descriptors: husks length in comparison to nut length, number of nuts per cluster, nut size, shape and colour of the shell, shell striping, size of pistil scar, size and shape of the kernel, appearance of kernel skin, size of inside cavity of the kernel. Phenological observations on flowering time and nut maturity were carried out in the Verduno site SAF5, SAF6 and TGdL plants.

The alleles of incompatibility of SAF7 were determined. In middle December the SAF7 plant was emasculated and afterwards two branches were isolated using paper bags. At the beginning of February the branches were taken and kept in laboratory to be manually pollinated. Pollens of different cultivars (TGdL, ‘Tonda di Giffoni’ and ‘Cosford’) were tested. After pollination female buds were kept in thermostatic cell and afterwards observed by fluorescence microscope following the protocol by Martin (1959).

In 2008 and 2009 years, three replications of 100 nuts per plant were also used to describe the following parameters: nut weight and diameter; roundness index of the nut; shell thickness; kernel weight

and diameter; homogeneity of kernel diameter (percentages of kernels including in three consecutive diameters); commercial and theoretical (excluding nuts with defects) percent kernel by weight; percentages of blanks and double kernels; blanching index expressed as percentage of kernels with pellicle removed over 75% (BI₁) and 50% (BI₂) of the surface after oven roasting at 160°C for 20 min.

2.3. *Sensory analysis*

All the sensory evaluations were conducted in a sensory analysis laboratory where the panelists were seated in individual testing booths. All the participants were previously trained for hazelnut tasting. Samples were supplied in coded (with a three-digit number) white plastic cups. Water was provided for palate cleansing.

Difference tests were performed using triangle test method, and the significance of the tests was determined from statistical tables (Meilgaard et al., 1999). In addition, a preference between the two samples concerning organoleptic quality was requested. Tests were designed depending on nuts availability of each accession. In 2008 a mixed sample of SAF5, SAF6, SAF7 and SAF8 nuts, was analysed with a bulk sample of TGdL. Two tests were performed using chopped raw and roasted kernel samples. The panel of judges consisted in 15 panelists. In 2009, single samples of SAF5, SAF6, SAF7 and SAF8, were compared with a bulk sample of TGdL as chopped raw kernels. The panel of judges consisted in 14 (SAF5) and 16 (SAF6, SAF7, SAF8) panelists.

2.4. *Microsatellite marker analysis*

DNA was extracted from 0.2 g of young catkins of each clone using the modified procedure described by Thomas et al. (1993).

Twenty-seven previously reported SSR loci were used to fingerprint all accessions surveyed: CaT-B107, CaT-B501, CaT-B502, CaT-B503, CaT-B504, CaT-B505, CaT-B507, CaT-B508 (Boccacci et al., 2005), CaC-A014a, CaC-B010, CaC-B020, CaC-B028, CaC-B029b, CaC-B113, CaC-C028, and CaC-C118 (Bassil et al., 2005a), CaC-A040, CaC-B014, CaC-B105, CaC-C115, and CaC-C119 (Bassil et al., 2005b), CaC-B011, CaC-C001a, CaC-C008, and CaC-C114 (Mehlenbacher et al., 2006), A611 and B606 (Gürçan et al., 2010a). PCR amplification was performed in a volume of 15 µL containing 40 ng DNA, 0.5 U Taq-DNA

polymerase (AmpliTaq Gold, Applied Biosystems Inc., Foster City, Calif.), 1.5 µL 10x PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 2 mM MgCl₂, 200 µM dNTPs and 0.5 µM of each primer. The PCR conditions included a initial denaturation step at 95 °C for 9 min., followed by 26 cycles of denaturation (30 s at 95 °C), annealing (45 s at 55 °C and 50 °C for CaT-B502), and extension (90 s at 72 °C). The final elongation step was at 72 °C for 30 min. The forward primers were labeled with a fluorochrome (6-FAM, HEX, NED or PET) and amplification products were analyzed using an ABI-PRISM 3130 Genetic Analyzer capillary sequencer (Applied Biosystems). Results of the run were then processed with GeneMapper software and allele sizes were estimated using the GeneScan-500 LIZ size standard (Applied Biosystems).

2.5. Field trial comparison

A field trial aimed at comparing TdB and TGdL in the same environmental conditions started in 2006 in Lu Monferrato (N 45°0', E 8°29', altitude 300 m a.s.l.), a hilly area located in the southern part of the Piedmont region. TdB plants used for the trial originated from the SAF5 and SAF6 plants located in the Verduno site. The hazelnut orchard had plant spacing of 5 x 4 m, with tree form training system, and no water supply.

Vegetative, productive and phenological traits were detected in 2007-2012 period. Three replications of five plants each were used to determine the following parameters: height of the tree, trunk circumference, number of suckers, productivity, time of female and male flowering, time of nut maturity. From 2009 to 2012, 100 nuts per plot were also used to describe the morphological and technological traits of the fruits, with the same methodology reported above.

2.6. Statistical analysis

All the numerical data were analyzed by ANOVA and Tukey's test using the software SPSS Statistics 20.0 (IBM, New York). A principal component analysis (PCA) was performed using data of 13 quantitative nut descriptors (shown in details in Tables 4 and 5) of the SAF and TGdL accessions.

3. Results

3.1. SSR analysis

Genetic analysis at 27 SSR hazelnut loci revealed the same genetic profile for all SAF accessions that corresponded to the genetic profile of the standard TGdL (Table 2).

3.2. Vegetative and phenological observations

The SAF accessions surveyed *on-farm* showed plants with medium or strong vigour, semi-erect habit and medium density of the shoots (Table 1). The suckers aptitude was medium, except for the accessions surveyed in Castelletto Stura: the numbers of suckers was very low in SAF148 and completely absent in SAF149. The productivity was medium or high in all accessions.

Phenological observations on flowering time carried out in Verduno, showed a great similarity between SAF5 and SAF6 accessions and TGdL1 plant. The flowering time was from the end of December to the half of January for male catkins, and from the half of January to the half of February for female flowers. However, for SAF accessions the peak bloom date was about 10 days earlier in comparison to TGdL1 in the same orchard. Also the nut maturity was earlier of about 10 days for the two SAF accessions in comparison to TGdL1. Nuts of the SAF accessions fell mostly (90%) in the first decade of August, while these of TGdL1 fell in the second decade.

The alleles of incompatibility of SAF7 resulted $S_2 S_7$ as those of TGdL.

3.3. Nut and kernel traits

Fruits of SAF accessions surveyed *on-farm* and TGdL clones (TGdL4 and TGdL5) showed involucre with no constriction and longer than nut length. Also, the colour of the shell (light brown), the presence of stripes (medium), and the size of the kernel (medium) did not show differences among accessions. The other nut traits that showed differences among accessions are reported in Table 3. The number of nuts per cluster ranged from 2-3 to 3-4. In SAF accessions nut size ranged from medium to medium-large, with a spheroidal or ovate shape of the nut, while the clones of TGdL had medium size and spheroidal shape of the nut. The size of pistil scar was generally small, but it was small to medium in SAF5, SAF6 and SAF8. The shape of the kernel was spheroidal for TGdL clones, and ovate for SAF accessions. The appearance of skin ranged from slightly-medium to strongly corky. The size of inside cavity of the kernel was small to medium in

TGdL clones and in SAF5, SAF7, and SAF11, while for the other SAF accessions was medium or medium to large.

The morphological parameters of the nut observed during 2008-2009 are reported in Table 4. Although SAF accessions did not show significant differences from TGdL, the roundness index of the nut and the shell thickness were the most relevant parameters able to characterize SAF accessions in comparison to TGdL. Values of roundness index ranged from 0.88 to 0.93 for SAF accessions and from 0.93 to 0.98 for TGdL. Shell thickness was larger for SAF accessions, ranging from 1.30 to 1.57 mm, in comparison to TGdL accessions, where ranging from 1.09 to 1.19 mm.

Among commercial traits, the percent kernel by weight of SAF accessions was significantly lower in comparison to TGdL (Table 5). Values of commercial percent kernel ranged between 39.09% and 41.58% for SAF accessions, while for TGL samples ranged between 46.99 and 48.98%. The presence of double kernels ranging from 4.33 to 8.83% in SAF accessions and was higher than that of TGdL, in which was lower than 1.50%. No significant differences were found for the percentage of blanks. The blanching after roasting gave good results in all samples. For TGdL the blanching index BI_1 was over 80.0% in all samples, while in SAF accessions ranged from 62.8% for SAF12 to 91.8% for SAF6.

In the PCA obtained from 13 quantitative nut descriptors, the first two components (PC1 and PC2) explained 78.0% of the total variation. PC1 accounted for 56.0% of variation and was mostly related with percent kernel, roundness index of the nut, homogeneity of kernel diameter, shell thickness and double kernels. The weight of the kernel was the trait that mostly influenced PC2 that accounted for an additional 22.0% of variation (Fig. 1a). SAF accessions were well discriminated from TGdL accessions (Fig. 1b).

3.4. Sensory analysis

Two triangle tests were carried out in 2008 on a mixed sample of SAF5, SAF6, SAF7 and SAF8 and a bulk TGdL sample. The test on chopped raw kernel was performed by 15 panelists. The correct identifications of the different sample was made by 11 panelists, so the test was considered highly significant ($P=0.001$). Among the 11 judges, 7 of them expressed an organoleptic preference for the TGdL sample, while 4 showed a preference for SAF sample. The second test performed on chopped roasted kernel gave a

result at the limit of significance ($P=0.10$). In this case, 8 out of 15 panelists made a correct identification giving a preference to the TGdL sample in 7 cases and to the SAF sample only in one case.

In 2009, a set of triangle tests on chopped raw kernel were carried out using single samples of SAF5, SAF6, SAF7 and SAF8 accessions in comparison to a TGdL bulk sample. All the tests performed gave a significant identification (Table 6). The panelists preference was assigned to the TGdL sample for more than 58.3% of cases, and for more than 90% for the SAF8-TGdL test.

3.5. Field trial comparison

The phenological observations carried out in the field trial confirmed that female flowering time and nut maturity were more precocious for TdB in comparison to TGdL. The time of female flowering as well as nut maturity was about 10 days earlier for TdB. In 2012, the 85% of the TdB nuts fell within the first decade of August against only the 45% of TGdL.

No significant differences were found between TdB and TGdL plants for vegetative and productive traits, although TdB was less vigorous, suckering and productive than TGdL (Table 7). However, TdB showed a higher cropping efficiency in comparison to TGdL.

The data from morphological analysis of the fruits showed a significant difference between TdB and TGdL for shell thickness that was greater for TdB in comparison to TGdL (Table 8). Both values of commercial and theoretical percent kernel by weight were significantly higher for TGdL in comparison to TdB (Table 9). In addition TdB had a significant higher presence of double kernels.

4. Discussion

DNA typing of hazelnut cultivars using molecular markers has become a useful method in recent years. Molecular markers, SSR in particular, were used to resolve cases of homonymy and synonymy, to fingerprint varieties and to search for the parents of cultivars (Boccacci et al., 2006; Gökirmak et al., 2009). In the specific case of the hazelnut cultivar TGdL, RAPD markers were used by Valentini et al. (2001) to determine the level of genetic diversity among clones, but genetic differences were not found indicating that this variety grown in Piedmont has a monoclonal origin. Although the most common molecular markers (such as SSR, RAPD, AFLP) are suitable for the identification of cultivars selected from reproductive

events, they are not efficient in discriminating among clones of a cultivar. Yet, short mutations at SSR loci are consistent with the stepwise mutation model proposed for microsatellite evolution (Jarne and Lagoda, 1996) and, although rare, can be observed by chance among clones and sports of a cultivar, the more loci are analyzed. In hazelnut, for example, a 2 bp (base pairs) discrepancy was observed between clones of ‘Santa Maria del Gesù’ (syn. ‘Nocchione’) and between clones of ‘San Giovanni’ (Botta et al., 2011). In grapevine, clonal mutations at SSR loci were noted among clones (Crespan, 2004; Vignani et al., 1996) and cultivars well-known to be synonyms (Akkak et al., 2007; Ibáñez et al., 2000).

In the present paper 27 SSR loci were analyzed in the SAF and TGdL accessions and no diversity was found among the individuals thus suggesting that the SAF selections are clones of TGdL. Yet, results of other observations and analysis showed the presence of important differences that lead to consider all SAF accessions as mutations of TGdL. The low and non suckering plants (SAF148 and SAF149) were closer to the other SAF accessions than to TGdL references (Fig 1b).

TGdL reference plants and SAF accessions showed differences primarily for phenological and nut characters. Considering the vegetative traits of the tree, only suckering aptitude distinguished the SAF accessions from TGdL, the former having lower suckering propensity than the latter (Table 2). This tendency was also observed in the field trial where TdB and TGdL were grown in the same climatic and cultural conditions, although the data were not statistically different (Table 7). Phenological observations conducted both in Verduno with SAF5 and SAF6 accessions compared to TGdL in the same orchard, and in the field at Lu Monferrato, showed that TdB is a more precocious cultivar (about 10 days), both for flowering and for fruit maturation. This trait is considered very interesting for hazelnut since most cultivars drop their nuts in September or even early October, when the occurrence of rains is very likely and negative both for harvesting and for nut quality.

Fruit traits can be influenced by environmental conditions, including climate and orchard management. Yet, some morphological traits as involucre length compared to nut length, roundness index, percentages of kernel by weight, and blanching ability, show a high degree of consistency, so they are appropriate for cultivar identification (Yao and Mehlenbacher, 2000). Qualitative fruits traits were slightly able to discriminate the two cultivars. Generally SAF accessions showed a bigger nut size with a more elongated shape and an ovate shape of the kernel in comparison to TGdL references (Table 3). The other

considered traits did not showed relevant differences. Results obtained by quantitative analysis of morphological traits of the fruits were more powerful in discriminating the two cultivars. The most relevant parameter able to characterize SAF accessions in comparison to TGdL was shell thickness, that was always larger for TdB (Table 4). This trait determined a lower percentages of kernel by weight for TdB (mean value of theoretical percent kernel for SAF accessions was 42.79%) in comparison to TGdL (48.45%). The other important traits able to characterize TdB in comparison to TGdL was the presence of double kernels (Table 5) that was always higher in TdB (mean values for SAF accessions was 7.14%). Data obtained from the *on-farm* evaluation were confirmed by those obtained from the field trial comparison. Also in this case, shell thickness (Table 8), commercial and theoretical percent kernel, and presence of double kernels (Table 9) showed significant differences between the two cultivar.

Double kernels result from synchronous development and fertilization of both ovules (Mehlenbacher et al., 1993). Most of hazelnut cultivars showed little level of double kernels but some, such as the Spanish cultivar ‘Barcelona’ and ‘Segorbe’ (Germain et al., 2001) and the Turkish cultivars ‘Tombul’ (Mehlenbacher et al., 1993) and ‘Kalinkara’ (Beyhan and Marangoz, 2007) had a relevant percentage of double kernels. This characteristic is considered a defect by the food industry, in particular for the use in confectionery as whole kernel (Garrone and Vacchetti, 1992). The percent kernel by weight is an important trait for Italian hazelnut producers since this parameter is used to determine the price of nuts and it was 3-5% lower in TdB than in TGdL. This means a 6-10% lower income that should be compensated by a higher yield. TdB is considered by farmers more productive than TGdL but the data of the field trial have not shown, so far, such a characteristic.

Results obtained from the sensory analysis performed by triangle tests of difference on chopped raw kernel showed that panelists were able to discriminate TdB accessions from the TGdL reference sample both when they were tasted mixed and when they were individually tasted, giving an overall preference for TGdL in most cases. Since after roasting and blanching sensory analysis was less effective in discriminating between TdB and TGdL, the difference in organoleptic quality has probably to be searched in the chemical and physical properties of the raw kernel.

In conclusion, given that the distinctive characters found in TdB are stable and maintained through propagation, as shown in the field trial, TdB could be considered a distinct cultivar from TGdL (ISHS,

2009). The practical problem is how to easily distinguish the two cultivars since the major difference is phenological. In this case it may help the analysis of further molecular markers such as AFLP or S-SAP, successfully used in similar cases of clonal variation (Scott et al., 2000; Stajner et al., 2009; Venturi et al., 2006). Single nucleotide polymorphisms (SNPs) are only recently identified in hazelnut (Bryant, et al., 2010) but they sequences are not yet available in literature. They may become in the future a new resource for the genetic identification of clones within a cultivar. The low and non suckering plants (SAF148 and SAF149) showed several traits of TdB but the stability after propagation of the suckering aptitude, which is the most distinctive character, is still under investigation. The non suckering aptitude is a desired trait and the accession SAF149 is of great interest from an agronomic point of view.

This is the first report demonstrating the presence of mutations of agronomical relevance within a monoclonal cultivar of hazelnut. Most fruit tree species, including hazelnut, are vegetatively propagated to maintain agronomically valuable genotypes. However, after many propagation cycles, clones accumulate phenotypic differences in agronomic traits and clonal diversity appears (Orive, 2001). This diversity can then be used to select the best clones or a new improved cultivar within a given variety. A recent study of the molecular polymorphisms generated along vegetative propagation (Carrier et al., 2012) through a genome-wide comparison of spontaneous grape 'Pinot noir' clones showed that only a small number of SNP and indel events are at the origin of clonal variation, while mobile elements of many families are involved in most polymorphisms, displaying the highest mutational event. This is certainly a relevant breakthrough in the understanding of origin of mutations that, with further studies, may provide breeding tools able to improve the best cultivars by artificially inducing mutations in specific genomic sequences.

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Table 1
Vegetative parameters of SAF accessions and TGdL clones (TGdL4 and TGdL5).

Accession code	Locality	Latitude	Altitude	Vigour	Habit	Density of shoots	Suckering	Productivity
SAF5	Verduno	N +44°40', E +7°56'	349 m	Strong	Semi-erect	Medium	Medium	High
SAF6	Verduno	N +44°40', E +7°56'	349 m	Medium	Semi-erect	Medium	Medium	Medium
SAF7	Biglini	N +44°42', E +7°58'	187 m	Strong	Erect	Medium	Medium	High

SAF8	Biglini	N +44°42', E +7°58'	187 m	Medium	Semi-erect	Medium	Medium	High
SAF11	Lequio Berria	N +44°36', E +8°04'	677 m	Medium	Semi-erect	Medium	Medium	Medium
SAF12	Lequio Berria	N +44°36', E +8°04'	677 m	Medium	Semi-erect	Medium	Medium	Medium
SAF148	Castelletto Stura	N +44°26', E +7°38'	447 m	Medium	Semi-erect	Medium	Weak	High
SAF149	Castelletto Stura	N +44°26', E +7°38'	447 m	Medium	Semi-erect	Medium	Absent	High
TGdL4	Cravanzana	N +44°34', E +8°07'	585 m	Medium	Semi-erect	Medium	Strong	Medium
TGdL5	Cravanzana	N +44°34', E +8°07'	585 m	Medium	Semi-erect	Medium	Strong	Medium

Table 2

Genetic profile at 27 SSR loci of the TGdL and SAF accessions studied in the present research.

	locus 1	locus 2
CaT-B107	136	154
CaT-B501	130	130
CaT-B502	187	191
CaT-B503	115	123
CaT-B504	173	185
CaT-B505	116	128
CaT-B507	186	192
CaT-B508	148	164
CaC-A014a	219	225
CaC-A040	236	246
CaC-B010	218	218
CaC-B011	154	154
CaC-B014	191	195
CaC-B020	283	285
CaC-B028	257	263
CaC-B029b	123	127
CaC-B105	159	159
CaC-B113	175	177
CaC-C001a	211	217
CaC-C008	198	198
CaC-C028	133	133
CaC-114	267	276
CaC-115	175	175
CaC-C118	182	182
CaC-119	260	260
A611	201	203
B606	275	277

Table 3

Nut and kernel qualitative traits for SAF accessions and TGdL clones (TGdL4 and TGdL5). (2007-2009 observations)

Accession code	Nuts/cluster	Nut size	Nut shape	Size of pistil scar	Kernel shape	Appearance of skin	Size of inside cavity
SAF5	3 to 4	Medium to large	Spheroidal	Small to medium	Ovate	Slightly to medium corky	Small to medium
SAF6	3 to 4	Medium to large	Ovate	Small to medium	Ovate	Medium to strongly corky	Medium to large

SAF7	2 to 3	Medium to large	Ovate	Small	Ovate	Slightly to medium corky	Small to medium
SAF8	3 to 4	Medium to large	Ovate	Small to medium	Ovate	Medium to strongly corky	Medium
SAF11	2 to 3	Medium	Spheroidal	Small	Ovate	Slightly to medium corky	Small to medium
SAF12	2 to 3	Medium to large	Spheroidal	Small	Ovate	Medium to strongly corky	Medium to large
SAF148	2 to 3	Medium	Ovate	Small	Ovate	Medium to strongly corky	Medium
SAF149	2 to 3	Medium to large	Spheroidal	Small	Ovate	Strongly corky	Medium to large
TGdL4	2 to 3	Medium	Spheroidal	Small	Spheroidal	Medium corky	Small to medium
TGdL5	2 to 3	Medium	Spheroidal	Small	Spheroidal	Medium corky	Small to medium

Table 4

Nut and kernel traits of SAF and TGdL accessions (mean values 2008-2009). Means within a column followed by with the same letter are not significantly different ($P \leq 0,01$; Tukey test).

Accession code	Nut weight (g)	Nut diameter (mm)	Roundness Index	Shell thickness (mm)	Kernel weight (g)	Kernel diameter (mm)	Kernel homogeneity (%)
SAF 5	2.72 ABC	19.33 ABC	0.93 AB	1.42 ABC	1.15 AB	13.75 AB	80.45 AB
SAF 6	2.81 AB	19.43 AB	0.89 B	1.33 ABCD	1.24 A	14.03 A	82.01 AB
SAF 7	2.37 CD	18.24 DE	0.88 B	1.34 ABCD	1.04 AB	12.68 B	79.08 AB
SAF 8	2.66 ABC	18.98 ABCDE	0.88 B	1.36 ABCD	1.17 AB	13.44 AB	83.57 AB
SAF 11	2.46 ABCD	18.42 BCDE	0.91 AB	1.52 AB	1.01 B	13.43 AB	84.66 AB
SAF 12	2.72 ABC	18.95 ABCDE	0.91 AB	1.57 A	1.14 AB	13.83 A	85.69 AB
SAF 148	2.55 ABCD	19.29 ABCD	0.90 AB	1.30 BCDE	1.09 AB	13.28 AB	75.66 B
SAF 149	2.85 A	19.68 A	0.93 AB	1.42 ABC	1.23 AB	14.19 A	81.85 AB
TGdL1	2.55 ABCD	18.67 ABCDE	0.97 AB	1.19 CDE	1.20 AB	14.03 A	84.51 AB
TGdL2	2.37 CD	18.31 CDE	0.96 AB	1.18 DE	1.15 AB	14.31 A	88.85 AB
TGdL3	2.40 ABC	18.36 BCDE	0.93 AB	1.19 CDE	1.19 AB	13.98 A	91.88 A
TGdL4	2.28 D	17.96E	0.96 AB	1.09 E	1.09 AB	13.95 A	88.36 AB
TGdL5	2.15 D	17.90 E	0.98 A	1.15 DE	1.04 AB	13.90 A	87.39 AB

Table 5

Commercial traits, nut and kernel defects of SAF and TGdL accessions (mean values 2008-2009). Means within a column followed by with the same letter are not significantly different ($P \leq 0,01$; Tukey test).

Accessions code	Commercial percent kernel (%)	Theoretical percent kernel (%)	Blanks (%)	Double kernels (%)	BI ₁ (%)	BI ₂ (%)
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SAF 5	39.09 B	42.37 DE	3.83	7.83 A	85.19 AB	94.80 AB
SAF 6	41.49 B	44.19 CD	3.83	6.83 AB	91.80 A	97.53 A
SAF 7	40.10 B	43.52 DE	2.00	6.83 AB	79.26 AB	88.67 AB
SAF 8	42.18 B	43.83 DE	2.83	8.83 A	83.95 AB	91.97 AB
SAF 11	39.79 B	41.03 E	3.83	4.33 ABCD	71.90 AB	83.66 AB
SAF 12	40.70 B	41.64 DE	1.50	8.33 A	62.79 B	79.12 B
SAF 148	40.11 B	42.87 DE	1.83	7.83 A	83.27 AB	92.05 AB
SAF 149	41.56 B	43.28 DE	4.00	6.33 ABC	82.06 AB	91.60 AB
TGdL1	46.21 A	46.99 BC	1.17	1.50 BCD	80.30 AB	93.09 AB
TGdL2	48.15 A	48.65 AB	0.88	0.67 CD	89.29 A	97.41 A
TGdL3	48.98 A	49.95 A	1.81	0.33 CD	91.72 A	97.35 A
TGdL4	46.39 A	47.94 AB	1.00	1.33 BCD	84.76 A	95.36 A
TGdL5	47.22 A	48.53 AB	2.22	0.00 E	90.39 A	98.33 A

Table 6

Results of triangle tests performed on chopped raw kernel samples of SAF accessions and TGdL (2009).

Samples	Correct identification of different sample	Significance	Preference for SAF sample	Preference for TGdL
SAF5 vs TGdL	11/14	0.001	4	7
SAF6 vs TGdL	13/16	0.001	5	8
SAF7 vs TGdL	12/16	0.001	5	7
SAF8 vs TGdL	11/16	0.01	1	10

Table 7

Comparison between vegetative and productive traits of TdB and TGdL in Lu Monferrato orchard (mean values 2012).

ns = not significant

Cultivar	Trunk area (cm ²)	Tree height (cm)	Number of suckers	Yield (Kg/tree)	Cumulative yield 2009-2012 (Kg/tree)	Cropping efficiency (g/cm ²)
TGdL	48.9	324.4	74.5	1.35	2.63	27.66
TdB	35.3	337.7	47.3	1.21	2.08	34.32
Significance	ns	ns	ns	ns	ns	ns

Table 8

Nut and kernel traits for TGdL and TdB detected in Lu Monferrato orchard (mean values 2009-2012).

ns = not significant * significant at $p \leq 0.05$

Cultivar	Nut weight (g)	Nut diameter (mm)	Roundness Index	Shell thickness	Kernel weight	Kernel diameter	Kernel homogeneity
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				(mm)	(g)	(mm)	(%)
TGdL	2.31	17.80	0.91	1.18	1.14	13.64	90.53
TdB	2.40	18.16	0.90	1.32	1.12	13.64	89.05
Significance	ns	ns	ns	*	ns	ns	ns

Table 9

Commercial traits, nut and kernel defects for TGdL and TdB observed in Lu Monferrato orchard (mean values 2009-2012). ns = not significant * significant at $p \leq 0.05$

Cultivar	Commercial percent kernel (%)	Theoretical percent kernel (%)	Blanks (%)	Double kernels (%)	BI ₁ (%)	BI ₂ (%)
TGdL	48.65	49.56	2.02	0.17	85.20	92.19
TdB	45.75	46.67	1.90	5.92	86.00	94.94
Significance	*	*	ns	*	ns	ns

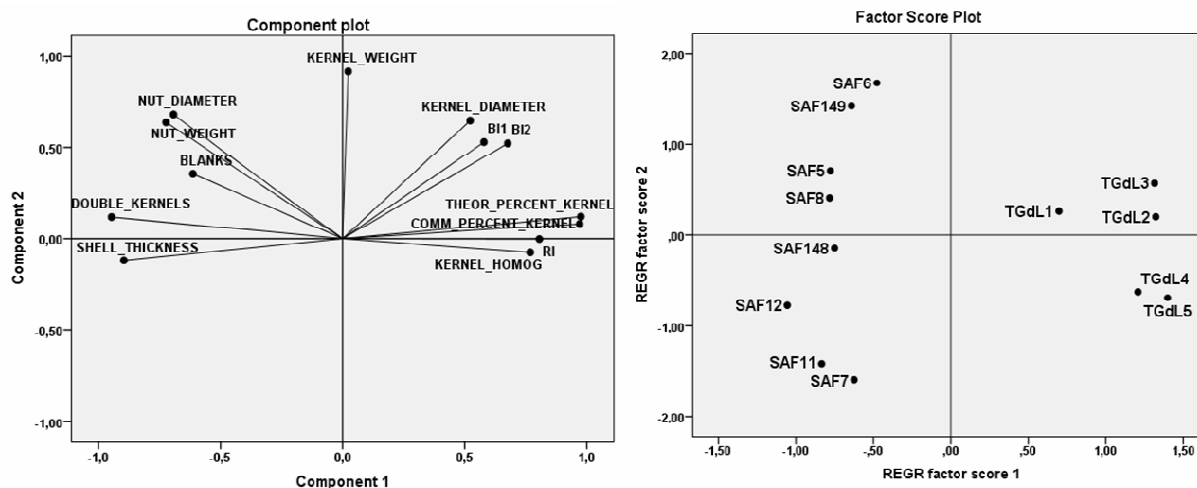


Fig. 1

Two-dimensional PCA based on the first two principal components (PC1 and PC2) generated by the analysis of quantitative nut characteristics of SAF and TGdL accessions: component plot (left) and scatterplot of factor scores (right)